The polyacrylic acid/modified chitosan capsules with tunable release of small hydrophobic guests

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Abstract

Nanocapsules (≤200 nm) with protection effect toward small hydrophobic guests (p-nitrophenyl laurate and acetylsalicylic acid, aspirin) and tunable release behavior have been fabricated through the layer-by-layer deposition of polyacrylic acid and modified chitosan. The dispersed particles of the loads pretreated with cationic surfactant, cetyltrimethylammonium bromide were used as template. The release profile was monitored through our own protocol involving fast cleavage of the substrate released and spectrophotometric control of the product. The shell permeability of the capsules and hence their protective effect may be tuned through the variation of the number of layers deposited, the sonication, and the adjustment of solution pH. Importantly, the dispersed loads serving as a template for the capsule fabrication may control their properties, including shell permeability.

Keywords capsule; layer-by-layer; polyacrylic acid; chitosan; release; carboxylic acid esters; acetylsalicylic acid

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1. Introduction

At present, the synthesis of polyelectrolyte micro- and nanocapsules attracts the attention of researchers all over the world due to their wide application in different areas, in particular in pharmaceutical, cosmetic, food, textile and agricultural industries [1-4]. Polyelectrolyte capsules are fabricated through alternating the pairs of oppositely charged polymers forming a hollow space and thin walls. The design and targeted modification of the capsule walls by varying the number of layers, the choice of shell material and the way of release of encapsulated compounds are of great importance from the viewpoint of controlling the characteristics and functions of the capsules. Among the various techniques for the formation of polyelectrolyte capsules, the strategy of layer by layer (LbL) deposition of oppositely charged polyelectrolytes first suggested by Decher et al appears to be in the foreground [5,6]. This approach has been successfully used for the encapsulation of antibodies for their targeted delivery to the affected cells [7], for the fabrication of metal nanoparticles with advanced sensitivity toward the laser radiation and magnetic fields [8,9], etc. Examples are available of using the LbL technique for the visualization of encapsulated dyes and probes by spectrophotometry and fluorescence spectroscopy [1]. There are variety of the LbL protocols, among which template methods [10] using the organic and inorganic cores [11-13], liposomes [14], and surfactants [15,16] should be notably mentioned. Each one of the methods has its own advantages, disadvantages and limitations, with the control of release of loaded substrates being the most important problem. The latter can be solved not only at the stage of capsule synthesis, but assumes the responsibility to external stimuli, e.g., changes in ionic strength [17] and pH [7, 18], the effect of light [19] and enzymes [20].

Polystyrene sulfonate, polyethyleneimine, polyacrylic acid, polymethacrylic acid, polyvinyl sulfate, and polyallylamine [21] were used as synthetic polyelectrolytes to form

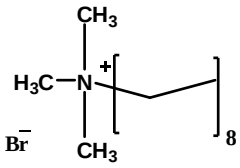
capsules. Nucleic acids, proteins [22] and polysaccharides [1,7], in particular alginic acid, chitosan, dextran sulfate, and carboxymethyl cellulose are favored among the natural polymers.

Previously [23], polyelectrolyte capsules based on polyacrylic acid and polyethyleneimine with hydrophobic carbonic acid esters inside have been synthesized in our research group. These substrates can be considered as models of water-insoluble drugs. The release of carbonic acid esters from the capsules can be monitored by means of registration of the spectral absorption band in the visible spectrum. In this paper, in order to increase the biocompatibility and hence the applicability of polyelectrolyte capsules for biological purposes the polyethyleneimine has been replaced by chitosan.

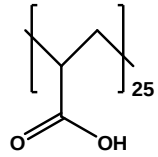
Chitosan is a partially deacetylated derivative of chitin. Chitosan possesses a number of unique physical and chemical properties, i.e. biocompatibility, biodegradability, antimicrobial activity, and the capability of chemical modifying to yield various derivatives [24, 25]. The experimental data on synthesis, investigation and applying of the polyelectrolyte complexes of chitosan with natural and synthetic polyanions are summarized in review [26]. In the literature, data on using chitosan in the synthesis of polyelectrolyte capsules are available [27-34], with the alginate [30,31] and dextran [27,20,33] often used as the polyanion for chitosan. It has been shown that the polymer characteristics such as molecular weight, degree of deacetylation, and the conformation of the macromolecule affect the properties of capsules formed. The modification of chitosan is aimed at the improvement of its solubility at neutral pH. The most known derivative of chitosan is N,N,N-trimethyl chitosan chloride obtained by the quaternization reaction [1,35,36]. Besides, derivatives with thiol groups [37] which provide opportunities for the formation of hydrogen bonds and the ways to further functionalization [37,38] are known.

Herein, N-palmitoyl-N-trimethylammonium-6-O-glycol chitosan (Fig. 1) was chosen as a polycation to form polyelectrolyte capsules. Good results in medicine delivery were previously obtained for this derivative of chitosan [39-41]. The aim of this work is the synthesis of

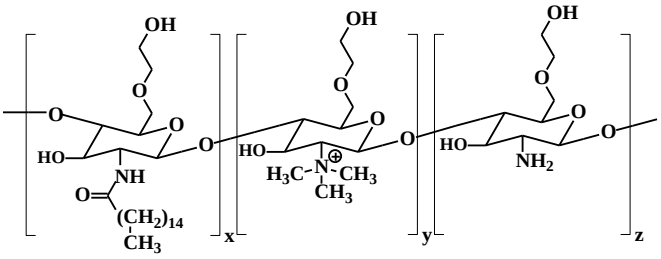
polyelectrolyte capsules by layer by layer deposition of the modified chitosan and polyacrylic acid (PAA) on the dispersion of p-nitrophenyl carboxylic acid esters and acetylsalicylic acid (aspirin) pretreated by cetyltrimethylammonium bromide (CTAB). The capsule stability over time and the influence of various factors (the number of layers, pH, ultrasonication) on the substrate release were evaluated.



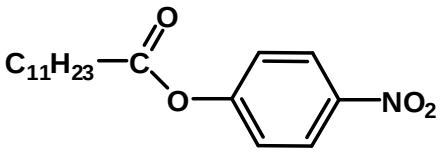
cetyltrimethylammonium bromide (CTAB)



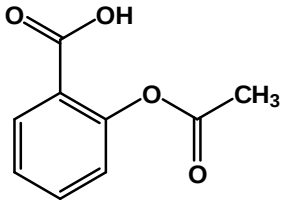
polyacrylic acid (PAA)



N-palmitoyl,N-trimethylammonium,6-O-glycol chitosan



p-nitrophenyl laurate (PNPL)



acetylsalicylic acid (aspirin)

Fig. 1. Substances used in the synthesis of polyelectrolyte capsules

Experimental Section

2.1. Materials.

Polyacrylic acid (M_w = 1800 g mol^{-1}, Aldrich) and chitosan synthesized according to the previously described technique [41,42] were used for the formation of polyelectrolyte capsules. Characteristics of chitosan are as follows: M_w = 10230 g mol^{-1}, M_n = 10010 g mol^{-1}, M_w/M_n = 1.79; dn/dC = 0.1491; mol % palmitoylation is 12.0; mol % quaternization is 6. The structure of chitosan was proved by the method of ^{1}H NMR spectroscopy (CD_3OD, Fig S1): $\delta_{0.85}$ = CH_3 (***CH₃***-CH_2-, palmitoyl), $\delta_{1.24}$ = CH_2 (-CH_2-***CH₂***-CH_2-, palmitoyl), $\delta_{1.55}$ = CH_2 (-***CH₂***-CH_2-CO-, palmitoyl), $\delta_{1.95}$ = CH_3 (***CH₃***-CO-NH-, acetyl-glycol chitosan), $\delta_{2.1\text{-}2.25}$ = CH_2 (-***CH₂***-CO, palmitoyl), $\delta_{2.7\text{-}3.1}$ = CH_3 [N***(CH₃)₂***-CH-, -dimethylamino-glycol chitosan], $\delta_{3.3}$ = CH_3

[N***(CH3)$_3$***-CH-, trimethylamino-glycol chitosan], $\delta_{3.4\text{-}4.2}$ = [-***CH***(OH)- and -***CH$_2$***-OH, glycol chitosan], $\delta_{3.27}$ = methanol and $\delta_{4.8}$ = water protons.

The p-nitrophenyl laurate (PNPL) (purity 99 %; Sigma-Aldrich) and aspirin was used as a substrate for the encapsulation. Cationic surfactant CTAB (Aldrich) was used for imparting the charge to neutral substrate molecule. The hydrolysis of encapsulated and free substrates was conducted in the solution of cetyldimethylhydroxyethylammonium bromide (CHAB) synthesized through reaction of 2-dimethylaminoethanol with hexadecyl bromide according to refs. [43, 44]. The structure of this compound was confirmed by elemental analysis, ^{1}H NMR and IR spectroscopy.

2.2. The synthesis of polyelectrolyte capsules.

First, 0.78 mL of 0.01 M ethanol solution of PNPL or aspirin was dropwise added to the 8 ml of 0.01 M CTAB solution with continuous magnetically stirring (1000 rpm). The dispersion obtained was centrifuged at 8000 rpm for 15 min and then decanted. Further, 8 mL of 1 mg/mL PAA aqueous solution was added to the precipitate and re-suspended under rapid stirring for 5 min. The procedure of centrifugation was repeated and the precipitate was separated. The same procedure was carried out with chitosan, i.e. 8 ml of the chitosan solution with a concentration of 1mg/ml was added to the precipitate, then rapidly stirred and centrifuged. During synthesis, solution pH was adjusted using acid or alkali.

Detailed technique of the capsule fabrication is described elsewhere [23]. Depending on the aim 3, 5 or 7 layers of polyelectrolytes were deposited. After the deposition of the last layer (PAA) the precipitate was separated, and 4 ml of bidistilled water was added to preserve it before further use.

2.3. The techniques for polyelectrolyte capsules investigation.

Dynamic light scattering measurements (DLS). The size and zeta potential of polyelectrolyte capsules were determined by photon correlation spectrometer of dynamic and electrophoretic light scattering Malvern ZetaSizer Nano (Malvern Instruments, UK). The source

of laser radiation was He-Ne gas laser with capacity of 10 mW and a wavelength of 633 nm. The angle of light scattering is about 173 degree. The accumulation time of pulses was 5-8 minutes. Signal analysis was carried out using single-board multi-channel correlator paired with PC equipped with a software package for estimating the effective hydrodynamic radius of the dispersed particles. Instrument accuracy is 5%.

Transmission Electronic Microscopy (TEM). The prepared dispersion of capsules were processed by using copper grids to adsorb the particles from the dispersion, then stained in 2.5 % uranyl acetate for 30 seconds and dried.[z1,z2] The specimen was observed under JEM 1200EX Transmission Electron Microscope (JEOL, Japan) operated at 80 kV. [45, 46]

Kinetic study. Hydrolysis of substrates occurred in alkali surfactant solution was monitored through changes in absorbance using a spectrophotometer «Specord 250» Plus (Germany) in thermostated cuvettes with pathway of 1.0 or 0.5 cm. The solution absorbance at a wavelength of 400 nm corresponds to the formation of p-nitrophenolate anion (the product of hydrolysis of PNPL), while absorbance at λ=299 nm was used to control the salicylate anion formed at the cleavage of aspirin. To estimate the rate of hydrolysis of encapsulated substrate the aliquot of aqueous dispersion of capsules was added into CHAB solution prepared using borate buffer at pH 9.2 or 10.0. Volume ratio and the thickness of the cuvette were chosen so that changes of the absorbance would be varied within the range of 0.2 to 1.5. The mixture was vortexed and placed in the spectrophotometer, whereupon the dependence of the absorbance at 400 nm for PNPL (or 299 nm for aspirin) on time was determined. The maximum absorbance (D_∞) corresponding to complete release of the substrate from the capsule was also evaluated, and the $\tau_{1/2}$ value was taken as the time at which the optical density was 0.5 D_∞.

For the case of "free" PNPL, the alkaline hydrolysis was carried out under the pseudo-first order reaction conditions. The initial substrate concentration was 2.0 to 8.0×10^{-5} M. The observed rate constants (k_{obs}) were determined from equation: $\lg(D_\infty - D_\tau) = -0.434 \cdot k_{obs} \cdot \tau + const$; here D_τ and D_∞ are the absorbance of solutions at point

τ and after completion of the reaction, respectively. The k_{obs} values were calculated using the weighed least-squares computing methods. The half-life values ($\tau_{1/2}$) were calculated using the formula $\tau_{1/2}= 0.69/k_{obs}$.

To obtain particles with uniform size, the sonication of the capsule dispersion in an ultrasonic bath Elmasonic S 15H (the operating frequency 35kHz, Germany) was performed for 30-40 min after each deposited layer.

2.4. Control of the load and loss of substrate.

The loss of PNPL at each stage of the capsules formation was determined spectrophotometrically. For this purpose, the washings and filtered solutions remaining after the deposition of each layer were adjusted to pH 11 with concentrated alkali to cleave the substrates. To accelerate the substrate hydrolysis, micellar catalyst CHAB was added in such an amount that its concentration in the solution reached approximately 0.003 M. After the constant value of absorbance of the solution at 400 nm was reached indicating that hydrolysis of PNPL is completed, the concentration of substrate in the sample was determined in accordance with Bouguer-Lambert equation C = D/ε l, where C is the concentration of the substance, l is the pathway, ε is extinction coefficient of ca.18000 L mol^{-1} cm^{-1}.

Further, the substrate loss at every stage and the total loss were determined taking into consideration the volume of the solutions filtered off. The similar algorithm was used to calculate the loss of aspirin. It was found that significant loss of the substrates has been observed during the encapsulation process that can reach ca. 52% of the initial amount, with the majority of the loss occurring at the stage of the isolation of the PNPL/CTAB complex (Table. SI).

Results and discussion

The technique of the deposition of polyelectrolyte layers over the substrate treated by cationic surfactant has been described in detail elsewhere [23], with the number of

polyelectrolyte layers varied from 3 to 7. The process of encapsulating the substrates is given in Fig. 2.

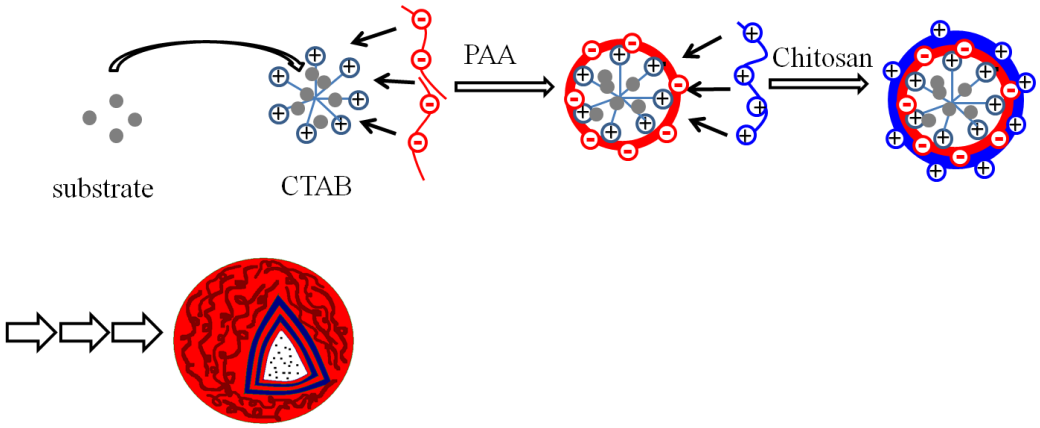


Fig. 2. The algorithm of fabrication of polyelectrolyte capsules with the LbL technique including the substrate treatment with cationic surfactant

3.1. The encapsulation of PNPL

The encapsulation process involves the deposition of the polyelectrolytes on PNPL previously treated with ionic surfactants. Herein, the charged substrate/surfactant complex is formed prior to the deposition of first layer of polyelectrolyte. This results in an increase in electrostatic interactions inside the system, which would determine the size of the capsule. The positively charged PNPL/CTAB complex (PNPL@CTAB) may be considered as a template for the capsule formation that determines the order of the layer deposition, starting with the oppositely charged polyanion of PAA. Due to electrostatic character of the layer interaction, this process may be successfully controlled by the measurements of electrokinetic potential. Data on the changes of zeta potential of the system in the process of p-nitrophenyl laurate encapsulation are given in Fig. 3. The recharging of the system is clearly observed when polyelectrolyte layers are deposited. The charge character is determined by the zeta potential value of an external layer, PAA or chitosan.

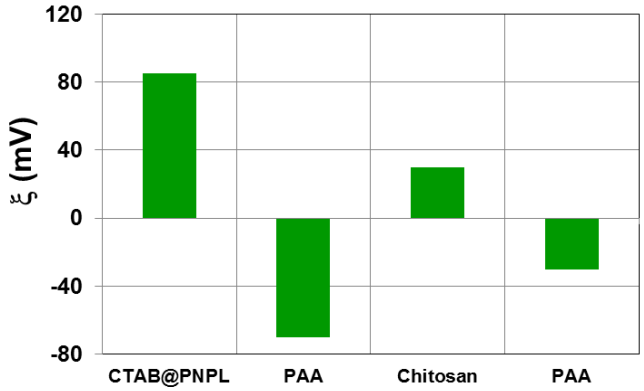


Fig. 3. Changes in zeta potential of the three-layered capsules fabricated through PAA and chitosan deposition with the use of PNPL@CTAB template, pH 6.

The size and the ability to release the substrate encapsulated are the principal properties of polyelectrolyte capsules that are determined not only by the choice of polyelectrolytes, but the conditions of synthesis and exploitation of capsules as well. The solution pH, the number of the deposited layers and involvement of ultrasonic treatment appear to be the most important factors. To elucidate the role of these factors, the variation of conditions of the PNPL encapsulation was attempted and the concomitant changes in capsule properties were monitored. The capsule permeability was verified through the protocol proposed in our work [23]. In particular, the ability of PNPL to serve as a probe was employed. The substrate from the capsule was released into the alkaline environment, in which it was quickly cleaved and a colored product was obtained. Half- time of the substrate cleavage is taken as the characteristic of the permeability of capsules. To evaluate the only substrate diffusion through the capsule shells, conditions should be chosen for its fast cleavage upon the release in bulk solution. Alkaline solutions of CHAB are used for this purpose as this surfactant is characterized by a high micellar catalytic effect toward the cleavage of ester bonds.

The first factor tested is the solution pH which is responsible for the polyelectrolyte charge density determining the electrostatic interactions between the layers. At pH>7 PAA is completely ionized, whereas the chitosan used exists as the polycation in a wide pH range. Thus, an effective interaction between the polyelectrolyte layers might be expected in neutral and alkaline media, which would provide a precondition of the compact shell formation. If the

capsules are fabricated without pH adjustment, the PAA may generate acidic conditions thereby demonstrating properties of the weak polyelectrolyte. This results in a much weaker interaction between the layers, and the capsule shell is much looser which would enhance its permeability for the substrate. Data on the effect of pH both on the size and permeability of the PAA/chitosan capsules are given in figs. 4,5.

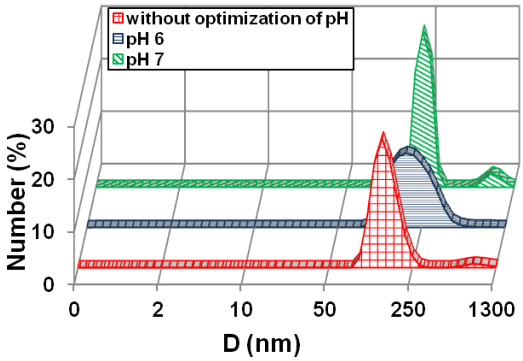


Fig. 4. Size distribution of three-layered capsules PAA/chitosan under different pH of the polyelectrolyte solution, 25^0C

The light scattering data testify that under spontaneous solution pH, 140-200 nm particles occur in the case of three-layered deposition, with high polydispersity and unsatisfactory correlation function observed. Capsules synthesized at pH 6 are characterized by monomodal size distribution with the prevalent population at 185 nm and the polydispersity index of 0.1. With the increase in pH to value of 7.0 the capsule size somewhat increases to 210 nm and the second peak appears indicating the small contribution of larger aggregates. Fig.5 visualized 3-layered capsules fabricated under fixed pH value of 6.0. The sizes of particles agree well with the DLS data, while demonstrate some higher polydidpersity.

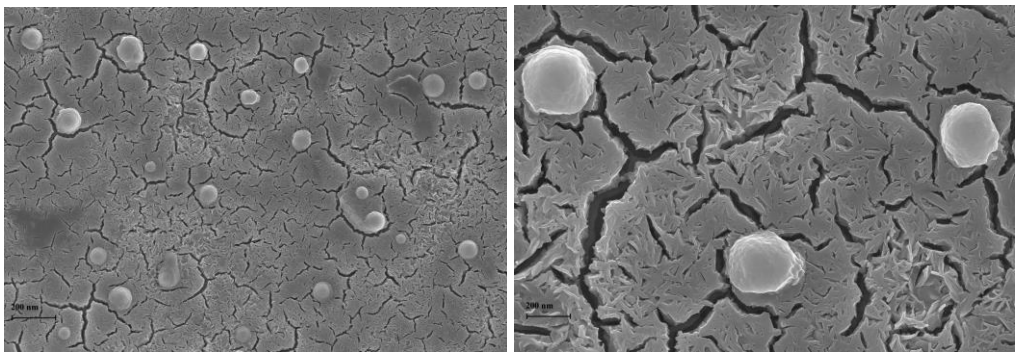


Fig. 5. SEM photo of 3-layered PAA/chitosan capsules fabricated at pH 6.0 with PNPL loaded.

Figure 6 shows that adjustment of solution pH is an important factor controlling the shell permeability. The half-life value of hydrolysis of PNPL penetrating through the three-layered capsules markedly increases (by ca. a factor of 3) in the case of capsules synthesized at pH value of 6.0 as compared to unfixed pH. Meanwhile, further variation of solution pH from 6 to 7 only slightly affects the release of the substrate. This probably evidences, that a looser shell is formed at spontaneous solution pH, since the conditions are lacking for the strong interaction of the layers to occur. An analogous influence of the pH on the properties of the capsules has been evidenced by us for capsules based on synthetic polyelectrolytes PAA and polyethyleneimine (PEI) [23]. It should be noted that the replace of the PEI by chitosan within the frame of the same protocol leads to the formation of three-layered capsules of similar sizes, but their permeability is nearly twice lower. Based on the data obtained one can conclude that pH 6.0 is optima for the synthesis of PAA/chitosan capsules.

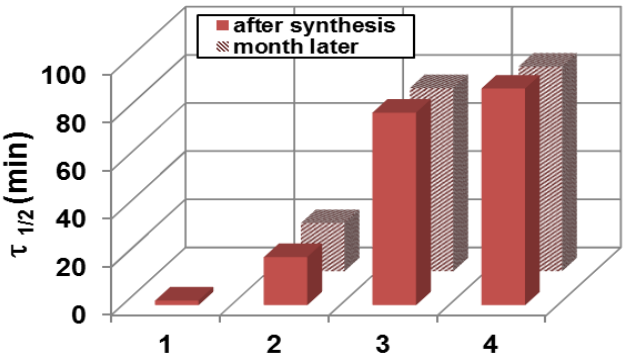


Fig. 6. The half-life values of hydrolysis of PNPL: “free” (1) and encapsulated in PAA/chitosan three-layered capsules synthesized with no pH optimization (2), at pH 6 (3), at pH 7 (4) immediately after synthesis and in a month; conditions are 3 мM CHAB, pH 9.2, 25°C

To emphasize the protection function capsules, the reactivity of free and encapsulated PNPL should be compared. It is known that hydrolysis rate of PNPL is strongly controlled by reaction conditions, such as pH, the presence of surfactants, their structure and concentration [23,

47]. Meanwhile all this tools are inactive in the case of encapsulated substrates due to protective effect of the capsule walls. In this case the capsule permeability becomes the major factor controlling its release and therefore the rate of hydrolysis. This can be evident when comparing the experimental data presented in Fig. 6. The hydrolysis rate of the encapsulated substrate appears to be mainly controlled by its diffusion through a polyelectrolyte shell rather than by the chemical process. It should be commented that capsules themselves can undergo changes under alkali conditions used for the fast substrate cleavage. At the same time, Fig. 7 illustrates that marked changes in the capsule size behavior including an increase in their polydispersity begin after 50 min exposing under aggressive conditions. Therefore those changes may only slightly affect the results of measurements. Moreover, as shown above, the increase in the polydispersity results in less compact packing the layer, thereby increasing the release of the substrate and understating the protecting effect.

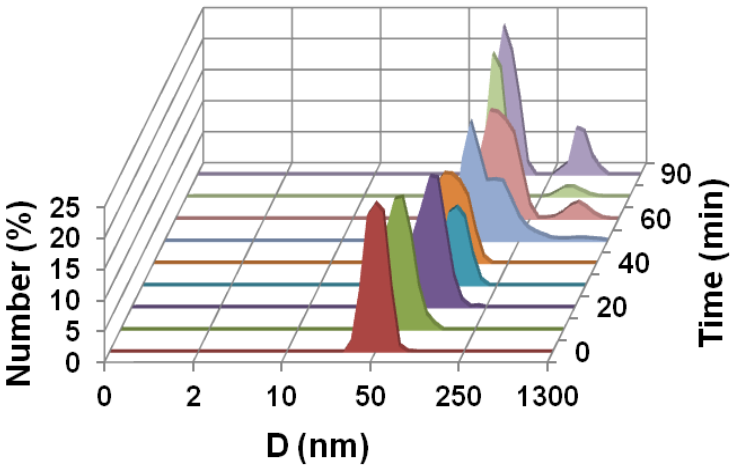


Fig. 7. The size distribution of three-layered capsules PAA/chitosan exposed for different period in 3 mM CHAB solution at 25^0C, pH 9.2.

Meanwhile, if the capsules are stored in bidistilled water, they keep their properties for a long time. We have observed the capsules size and their ability to release the substrate for a month (Fig.6). These properties remained unchanged, which means they are stable enough. We

have also shown that when PAA/chitosan capsules with PNPL are kept in weakly acidic media the interaction between polyelectrolyte layers did not disturb and capsules remain stable as well.

One more significant tool controlling the shell permeability is ultrasonic treatment that may promote to obtain disperse particles of smaller size. We tested this effect on the PAA/chitosan capsules fabrication assuming that sonication of each layer will result in an increase of the packing density of the polyelectrolyte molecules, and make it possible to obtain capsules of smaller size. However, sonication appeared to exert only slight impact both on capsule sizes and their permeability (Figs. 8, 9).

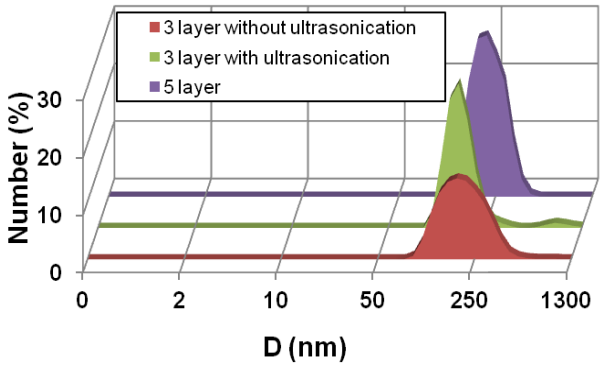


Fig. 8. Size distribution of PAA/chitosan capsules depending on the number of deposited layers and sonication: 25^{0}C, pH 6.0.

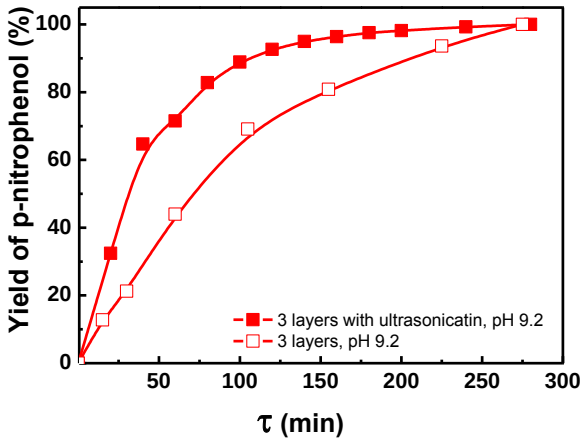


Fig.9. The release of PNPL from 3-layered PAA/chitosan capsules fabricated at pH 6.0 with and without sonication; reaction bulk conditions: 3 mM CHAB; pH 9.2; 25 ^{0}C.

The key factor determining the capsule permeability is documented to be the number of deposited polyelectrolytes layers. Increase in the number of layers in PAA/chitosan capsules with loaded PNPL results in some growth in particle size (Fig. 8) and increase in half-life time of the substrate hydrolysis (Fig. 10). However, the difference in half-life values for PNPL loaded in three - and seven-layered capsules does not exceed 30%, whereas in the case of PAA/PEI capsules formed under the same conditions, much more pronounced differences are observed.

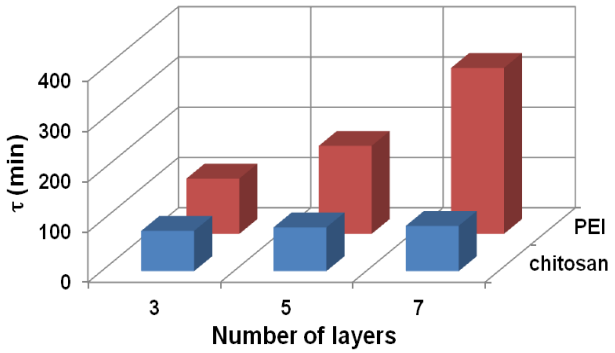


Fig. 10. The half-life values of hydrolysis of PNPL loaded into PAA/chitosan and PAA/PEI capsules fabricated at pH 6 with varied number of layers; reaction bulk condition: 3 mM CHAB; pH 9.2; 25 ^{0}C.

The following key points should be emphasized in this connection: (i) two sets of factors should be differentiated that control the PNPL release from the capsules, i.e. those of large-scale effect (through pH adjustment) and more delicate one (through sonication or the variation of layer deposited); (ii) unlike the PAA/PEI capsules the more uniform structural and release behavior is probably observed in the case of PAA/chitosan capsules regardless of the conditions used. This simplifies the protocol of capsule fabrication, allowing the sonication and multi-layering to be omitted.

The protocol developed can be applied to other uncharged low molecular weight substrates, in particular for encapsulating of water insoluble medicine and diagnostic agents.

3.2. The encapsulation of aspirin

Aspirin (acetylsalicylic acid) has been widely used for more than 100 years due to its analgetic, antipyretic, anti-inflammatory and antiaggregate effects. However, this medicine has

a number of significant limitations. Thus, the long-time administration of aspirin irritates the gastro-intestinal tract. This preparation is poor soluble in water. Besides, it may degrade with acetic and salicylic acids formation. The process of aspirin hydrolysis may be accelerated in the presence of enzymes or nucleophiles. Thus, the bimolecular constant of alkaline hydrolysis is equal to 14.2-15.6 M^{-1} min^{-1} (37°C) according to [48]. The scheme of the process is given in SI (Fig. S2).

The therapeutic characteristics of aspirin loaded into polyelectrolyte capsules can be dramatically improved due to (i) obtaining its nanoscale dispersion and thereby improving the pharmacokinetic properties, (ii) increase its hydrolytic stability, (iii) providing a prolonged effect. On the basis of polyacrylic acid and chitosan, three-layered capsules with loaded aspirin were synthesized by the LbL technique. The process of substrate encapsulation and release was controlled by spectrophotometry at 299 nm wavelength that corresponds to the absorption of salicylic acid, the product of aspirin alkaline hydrolysis. Changes in absorbance of the solution due to the release of the substrate from the capsules are determined by two factors: the diffusion of aspirin through the capsule shell into the bulk phase and the rate of hydrolysis. As stated above, the hydrolytic cleavage of ester bonds, and in particular, hydrolysis of aspirin may be significantly accelerated by increasing pH or adding cationic surfactant [49]. In order to increase the diffusion contribution and make it dominant, the following solution was used: borate buffer (pH 9.2), 0.01M NaOH (pH 12) in the absence and in the presence of 0.003 M CHAB. When comparing the half-life values of hydrolysis of the free and the encapsulated aspirin, conclusion can be drown on protective properties of the capsules.

Aspirin was pre-treated by CTAB as described in the experimental part. Capsules obtained at pH 6.0 by the step-by-step deposition of chitosan and PAA on treated medicine are characterized by a hydrodynamic radius of 150 nm and a polydispersity index of 0.6. In some experiments there appear particles of a larger size, though their contribution is insignificant. The DLS data in terms of size distribution are given in Fig.11. The loss of aspirin in the

encapsulating process amounted to approximately 60%. The time dependence of the yield of aspirin hydrolysis products are given in Fig. 12 and in Table 1.

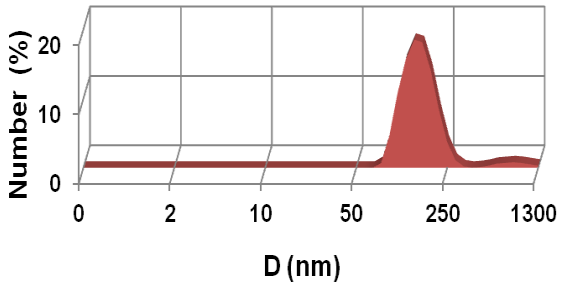


Fig. 11. The hydrodynamic diameter of three-layered PAA/chitosan capsules fabricated at pH 6.0 with loaded aspirin; 25^0C.

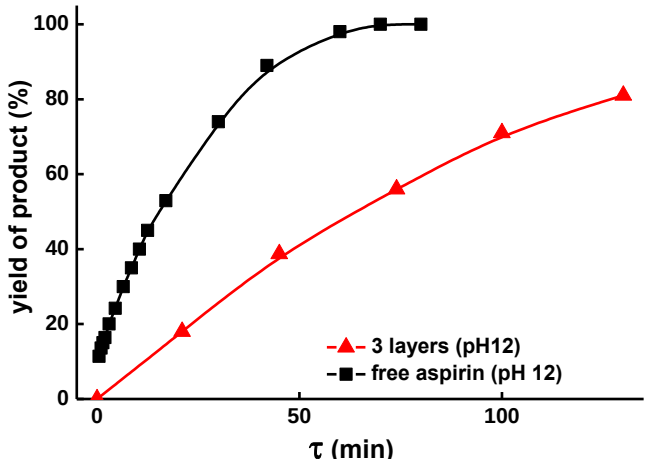


Fig. 12. Half-life values of hydrolysis of aspirin, free and encapsulated in three-layered PAA/chitosan capsules; bulk conditions: 0.01M NaOH; pH 12, 25^0C.

Table 1. The half-life values of aspirin hydrolysis in different conditions, i.e. free and loaded PAA/chitosan capsules

	$\tau_{1/2}$, h pH 9.2	$\tau_{1/2}$, h CHAB; pH 9.2	$\tau_{1/2}$, h 0.01M NaOH; (pH 12)	$\tau_{1/2}$, h CHAB; NaOH; pH 12
Aspirin loaded in 3-layered capsules	30	9.0	1.3	0.7
Free aspirin	25	4.0	0.1	

The half-life values for free aspirin in 0.01M NaOH solution is about 5 minutes, and for drug encapsulated in three-layered capsules in the same conditions is 75 minutes, while that in CHAB borate buffer is 9 hours, and in borate buffer is 30 hours. The more aggressive bulk solution (high pH value, the presence of cationic surfactants), the more the hydrolysis rate of the encapsulated substrate is determined by its diffusion through the shell and the greater the time difference and a half-encapsulated aspirin free (table1).

When comparing the characteristics of capsules based on PAA/chitosan containing PNPL and aspirin a number of several significant differences can be observed. (1)The three layered capsules formed at pH 6.0 with PNPL are characterized by a narrower size distribution than those containing aspirin; (2) Capsules with aspirin are less permeable for the substrate than those containing PNPL. It may be assumed that the acidic group of aspirin interacts with layers of shell through hydrogen bonds or other contributions, which prevents its release. (3) At the same time, upon the prolonged storage of the PAA/chitosan capsules (for one month) in bidistilled water, the amount of PNPL in the bulk solution is quite insignificant, whereas aspirin diffuses in appreciable quantities.

Thus, protocol for layer-by-layer deposition of polyacrylic acid and modified chitosan was developed aimed at the encapsulation of low molecular weight substrates, namely p-nitrophenyl laurate and acetylsalicylic acid, aspirin. Nanocapsules of ≤200 nm have been fabricated with the use of dispersed substrate particles pretreated with cationic surfactant as a template. The release behavior was monitored through our own protocol involving fast cleavage of the substrate penetrating in bulk solution and spectrophotometric control of the products, p-nitrophenolate anion at 400 nm or salicylic acid at 299 nm. The shell permeability of the capsules and hence their protective effect may be tuned through the variation of the number of layers deposited, the sonication, and the optimization of solution pH. The nature of load is shown to control the capsule properties including shell permeability.

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Supporting material

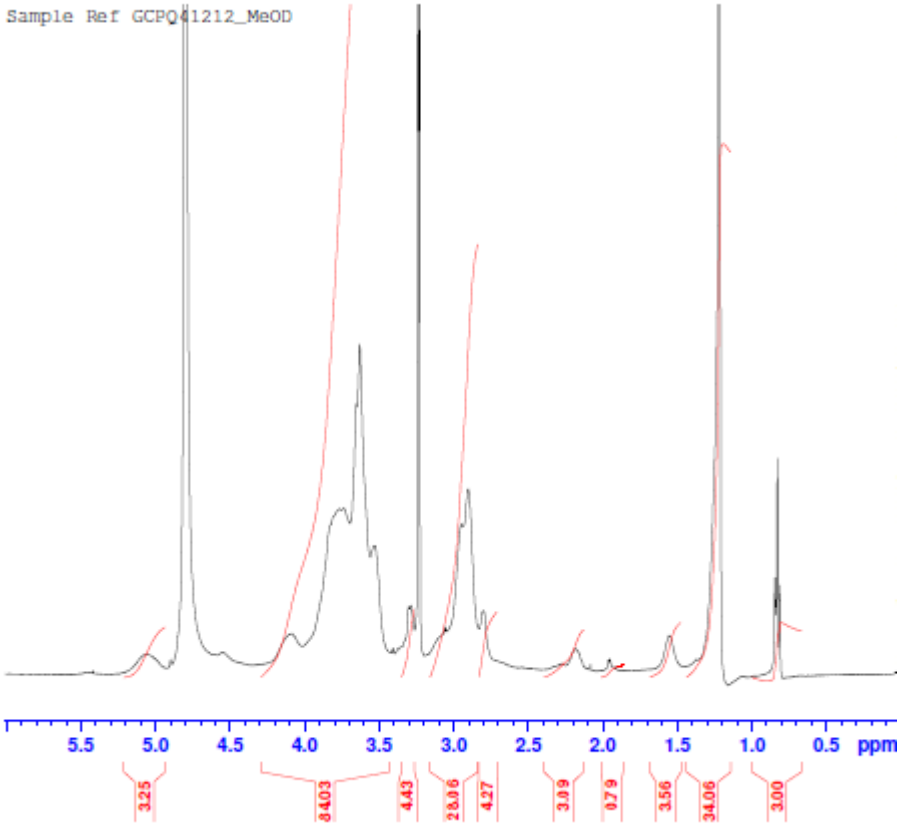

Fig S1. The ^{1}H NMR spectrum (600 MHz, CD_3OD, 303K) of the modified chitosan.

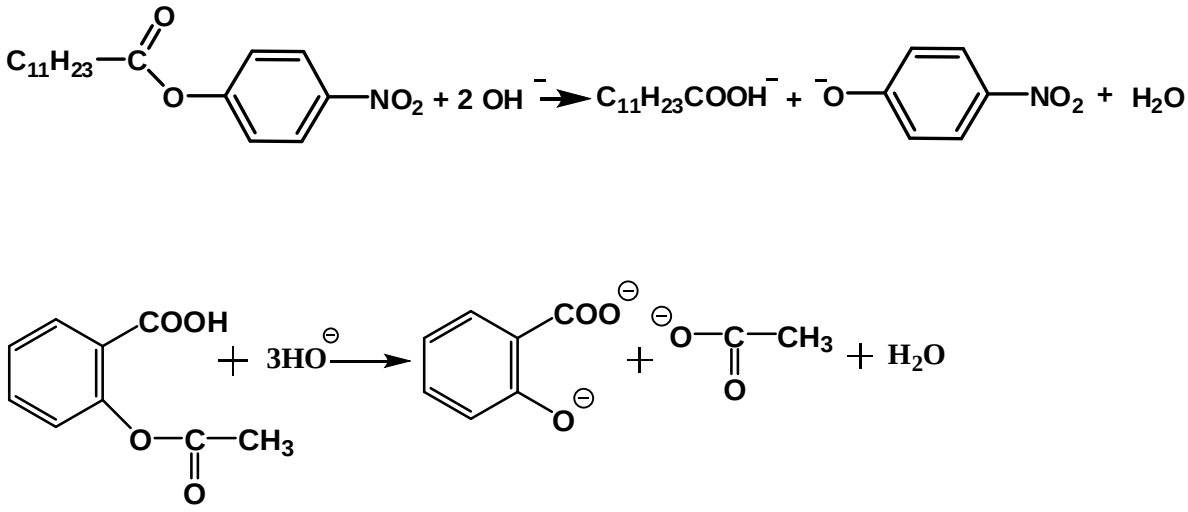

Fig S2. Schematic representation of the hydrolysis of PNPL and aspirin

Control of the substrate loading (three-layered capsules, pH 6.0, without ultrasonication).

Table S1. The monitoring of unloaded substrates in the supernatant fractions after each cycle of polyelectrolyte deposition*

Stage	Mass of PNPL in supernatant fraction, g	Unloaded PNPL, %	Mass of aspirin in supernatant fraction, g	Unloaded aspirin, %
The PNPL@CTAB preparation	0.0009	36	0.011	44
The PAA deposition (1-layered capsule)	0.0002	8	0.0030	12
The chitosan deposition (2-layered capsule)	0.0001	4	0.0010	4
The PAA deposition (3-layered capsule)	0.0001	4	0.0003	1
Total	0.0013	52		61

*Initially, 0.0025 g of PNPL or 0.025 g of aspirin was solubilized in 0.01 M CTAB solution, Amount of the unloaded substrate was spectrophotometry controlled after the completion of each coating procedure according to the protocol given in the Experimental section.